

Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa

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Background Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are highly effective tools for controlling malaria transmission in Africa because the most important vectors, from the *Anopheles gambiae* complex and the *A. funestus* group, usually prefer biting humans indoors at night.

Methods Matched surveys of mosquito and human behaviour from six rural sites in Burkina Faso, Tanzania, Zambia, and Kenya, with ITN use ranging from 0.2% to 82.5%, were used to calculate the proportion of human exposure to *An. gambiae sensu lato* and *An. funestus s.l.* that occurs indoors (π_i), as an indicator of the upper limit of personal protection that indoor vector control measures can provide. This quantity was also estimated through use of a simplified binary analysis (π_i^B) so that the proportions of mosquitoes caught indoors (P_i), and between the first and last hours at which most people are indoors (P_{in}) could also be calculated as underlying indicators of feeding by mosquitoes indoors or at night, respectively.

Results The vast majority of human exposure to *Anopheles* bites occurred indoors ($\pi_i^B = 0.79\text{--}1.00$). Neither *An. gambiae s.l.* nor *An. funestus s.l.* strongly preferred feeding indoors ($P_i = 0.40\text{--}0.63$ and $0.22\text{--}0.69$, respectively), but they overwhelmingly preferred feeding at times when most humans were indoors ($P_{in} = 0.78\text{--}1.00$ and $0.86\text{--}1.00$, respectively).

Conclusions These quantitative summaries of behavioural interactions between humans and mosquitoes constitute a remarkably consistent benchmark with which future observations of vector behaviour can be compared. Longitudinal monitoring of these quantities is vital to evaluate the effectiveness of ITNs and IRS and the need for complementary measures that target vectors outdoors.

Keywords Malaria, plasmodium, vector, transmission, exposure, behaviour

Introduction

Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are recognized as the most cost-effective methods for preventing malaria transmission by indoor-biting mosquitoes.^{1,2} The success of these interventions relies on their ability to repel or kill endophagic (indoor feeding) mosquitoes, thus providing direct personal protection against exposure to bites, as well as reducing adult mosquito survival and human-feeding frequency.^{3–10} The major malaria vectors of sub-Saharan Africa are *Anopheles gambiae* Giles and *An. arabiensis* Patton, which are members of the *Anopheles gambiae sensu lato* species complex, and *An. funestus* Giles, of the *An. funestus s.l.* species group.¹¹ These highly efficient vector species are generally considered to predominantly prefer feeding indoors (endophagy) at night (nocturnally), with peak biting activity typically occurring between midnight and the early hours of the morning, when most people in sub-Saharan Africa are asleep indoors.¹²

However, high coverage rates of ITNs or IRS can dramatically alter the composition of vector populations.^{13–20} Consequently, biting activity of the persisting residual populations tends to be more evenly distributed across the night because mosquitoes feeding indoors in the middle of the night are selectively suppressed.^{18–20} Together with emerging resistance to pyrethroids,²¹ the only class of existing insecticides suitable for use on ITNs, it is the host-seeking patterns of such residual vector populations that define the limit of how much malaria control can be attained with ITNs or IRS or both, as well as the ideal properties of complementary vector-control measures.^{22–25}

With so few reports with which to compare contemporary observations of changing vector behaviour^{18–20} and those that may occur in the near future, it is important to examine existing data to get a broader appreciation of the range of values for the proportion of human exposure to insect vectors of disease that has occurred indoors in sub-Saharan Africa. Conventional indices of behavioural patterns of malaria vectors can substantively underestimate the potential protection provided by ITNs against exposure because they do not consider human indoor and outdoor movements.²⁶ In the study described here, records of indoor and outdoor mosquito biting distributions from 10 *Anopheles* populations at six rural sites in Africa have been combined with surveys of the times at which humans enter and leave their houses each night, to understand how much can reasonably be expected from the ongoing scale-up of ITNs and IRS.^{22–26}

Methods

Study sites

Data were obtained from two multi-country studies spanning six rural sites in southern, eastern, and central Africa (Figure 1; Table 1). Included in this analysis are two sites in Burkina Faso (Oubritenga and Kourweogo) and two in Tanzania (Ulanga and Rufiji) that were in the Malaria Transmission Intensity and Mortality Burden Across Africa (MTIMBA) study conducted between 2001 and 2004, together with one site in Zambia (Luangwa) and one in Kenya (Rarieda) that were in the Malaria Transmission Consortium (MTC) study conducted in 2009 and 2010. The two sites in Burkina Faso had little coverage with any vector control measure at the time of the MTIMBA study, the two Tanzanian sites had low coverage with nets, and the sites in Zambia and Kenya that were in the MTC study had high coverage with ITNs. None of the sites was covered by an IRS programme. However, in the case of the Kenyan site, an incremental impact of IRS on malaria transmission was observed nearby when the Rachuonyo district was sprayed with the synthetic pyrethroid lambda-cyhalothrin.²⁷ None of the houses in which human landing collections (HLCs) were conducted had been sprayed with any residual insecticides.

Mosquito behaviour surveys

Mosquito biting rates were observed hourly during the night both indoors and outdoors through the human-landing catch (HLC) method,²⁸ with collectors using an aspirator and torchlight to catch mosquitoes landing on their exposed legs. At the MTIMBA study sites, except for Ulanga, two collectors interchanged their positions between indoors and outdoors every hour, and the pair was replaced by a second pair of collectors after the sixth hour. At the MTC study sites and the Ulanga site, a pair of collectors (one collector stationed indoors and one outdoors) did the collection throughout the night for 45 minutes every hour, with a 15-minute break.²⁹ The HLC exercise began at 18:00 hours in Rarieda and Rufiji, 19:00 hours in Lupiro and Luangwa, and 20:00 hours in Oubritenga and Kourweogo. Human-landing catch surveys finished at 06:00 hours in Rarieda and Rufiji and at 07:00 hours in all of the other sites.

Human behaviour surveys

Surveys of human behaviour were used to determine which hours residents spent indoors and outdoors at night. The MTIMBA and MTC studies used different methods for this. In the MTIMBA study, direct



Figure 1 Map of Africa showing locations of study sites

observations were recorded by a field worker who sat in a randomly selected compound and recorded the number of people who were awake at hourly intervals from 6.00 pm until all of them retired indoors. A similar procedure was used in the same compound from 4.00 am to 6.00 am on the following morning. In the MTC study, four questions were incorporated into standard cross-sectional malaria indicator survey questionnaires asking when, to the nearest hour, the respondent went indoors for the night, went to bed to sleep, awoke in the morning, and left the house in the morning.

Data analysis

Several studies have calculated the average proportion of human bites by a given vector population that occurs indoors in the absence of any protective

measure such as an ITN (π_i).^{18,19,26,30} This parameter limits the maximum possible degree of personal protection that any exclusively indoor measure can provide, and therefore the consequent level of indirect protection achieved through community-wide suppression of mosquito longevity, feeding frequency, and access to humans.^{24,31,32} This epidemiologically critical upper limit for personal protection³³ was initially calculated (Figure 3) by weighting the mean indoor and outdoor biting rates for each hour of the night by the proportion of humans reporting to have been indoors and outdoors, respectively, at that time.³⁰ In order to facilitate consistent mathematical description of this calculation, a sequence of 24-hour-long intervals is defined that begins at 18:00 hours on the conventional 24-hour clock, so that $t=0$ corresponds to the period from 18:00 to 19:00 hours and

Table 1 Description of study sites^a

| Site | Geographical coordinates | Duration | Study | Intervention(s) | ITN Use ^b | Dominant vector species | | Reference |
|-------------------------|--------------------------|----------|--------|-----------------|----------------------|-------------------------------------|--------------------------------------|-----------|
| | | | | | | <i>Anopheles gambiae sensu lato</i> | <i>Anopheles funestus sensu lato</i> | |
| Kenya Rarieda | 0.18 S 34.40 E | 2009 | MTC | ITNs | 82.5% | <i>An. arabiensis</i> | <i>An. funestus sensu stricto</i> | 14, 48 |
| Zambia Luangwa | 15.13 S 30.20 E | 2009–10 | MTC | ITNs | 66.0% | <i>An. quadrimaculatus</i> | <i>An. funestus s.s.</i> | 30 |
| Tanzania Rufiji | 7.95 S 38.98 E | 2002–04 | MTIMBA | ITNs | 25.4% | <i>An. gambiae s.s.</i> | <i>An. funestus s.s.</i> | 61 |
| Tanzania Ulanga | 8.35 S 36.67 E | 2002–04 | MTIMBA | ITNs | <20% | <i>An. gambiae s.s.</i> | <i>An. funestus s.s.</i> | 62 |
| Burkina Faso Oubritenga | 12.73 N 1.44 W | 2002–04 | MTIMBA | ITCs and ITNs | 0.6% | <i>An. gambiae s.s.</i> | <i>An. funestus s.s.</i> | 39 |
| Kourweogo | 12.73 N 1.75 W | 2002–04 | MTIMBA | ITNs | 0.2% | <i>An. gambiae s.s.</i> | <i>An. funestus s.s.</i> | |

^aITN, insecticide treated net; ITC, insecticide treated curtains; MTC, Malaria Transmission Consortium; MTIMBA, Malaria Transmission Intensity and Mortality Burden Across Africa

^bProportion of children <5 years old who reported using an ITN during the night before the survey.

$t=1$ to the period from 19:00 to 20:00 hours, and continuing through to $t=23$ for the period from 17:00 to 18:00 hours.³⁰ The proportion of human exposure to bites by a given vector population that occurs when residents are both indoors and sleeping or trying to sleep (π_s) was calculated similarly to π_i , using the same denominator estimate of total indoor and outdoor exposure, but a numerator that is the sum of the products of the mean indoor biting rates and the estimated proportions of humans reporting to have gone to bed to sleep for each hour of the night.³⁰

The proportion of exposure to mosquito bites of unprotected individuals that occurs indoors was also estimated, in a more simplified binomial fashion (π_i^B), so that it could be analyzed through logistic regression^{18,19,30} using generalized linear models (GLMs) specifically designed to quantify the influence of categorical or continuous independent variables upon binary dependent variables (Table 2).³⁴ The nightly interval that is considered as normally spent indoors was defined as beginning at the first (f) and ending at the last (l) hour when the majority of people were indoors, so that π_i^B could be calculated simply as the total number of mosquitoes caught indoors during that period, divided by the sum of this total and the total caught outdoors before and after this interval.³⁰

In order to more clearly interpret the estimates obtained, two underlying determinants of π_i^B were also calculated exactly as recently described.³⁰ These were: (i) the propensity of vectors to feed indoors, which is reflected in the proportion of all mosquitoes caught that were captured indoors (P_i); and (ii) the propensity of vectors to feed at times when people are indoors, which is reflected in the proportion of all mosquitoes caught that were captured during hours when the majority of people were indoors (P_{in}). These crude binomial estimates of P_i , P_{in} , and π_i^B allowed statistical comparisons to be made through logistic regression, using generalized linear models (GLM) with a logit link function and binomial distribution³⁴ for these binary outcomes (PASW Statistics, version 18; SPSS, Chicago, IL). Comparisons were made across categorical explanatory variables of site and species for tendency towards endophagy and nocturnal activity. Vector preference for both feeding indoors (P_i) and at times when most humans were indoors P_{in} was compared with the null hypotheses (P_i or $P_{in}=0.5$). The first (f) and last (l) hour during which most of the human population was indoors were estimated separately for each site on the basis of the surveys of human behaviour described above.

Protection of human subjects and ethical approval

Ethical clearance for the study was obtained from local ethical review bodies. Humans participating in the HLC exercise were made aware of the study procedures and risks involved in their participation. Necessary

precautions were taken, including regular screening for malaria parasites and prompt treatment of positive cases based on the prevailing guidelines for treating malaria. In Rarieda and Luangwa, collectors were provided with malaria prophylaxis with mefloquine (Lariam) and atovaquone–proguanil hydrochloride (Malarone), respectively. Before visits were made to households for human behaviour surveys, permission was sought from the appropriate local authorities.

Results

Vector behavioural patterns differed considerably among the study locations and vector taxa, with peaks of biting activity occurring at various times from just after dusk to just before dawn (Figure 2). Biting rates that were clearly higher indoors than outdoors were not as ubiquitous as expected, and were found for only four of the *An. gambiae s.l.* populations and one of the *An. funestus s.l.* populations. Figure 2 illustrates a substantial degree of diversity in human and mosquito behaviour across Africa. The amount of time that residents spent indoors during the night varied from 8 hours in Ulanga to 12 hours in Rarieda.

Despite the considerable diversity manifested in the 10 vector populations and the 6 human populations illustrated in Figure 2, Figure 3 depicts a remarkably consistent picture in terms of the generally high proportions of human exposure to mosquito bites that occur indoors. Although no data describing when residents slept (rather than merely spent indoors) were available for most of the study sites, these data were available for both Rarieda and Luangwa, for which the more directly relevant proportion of exposure occurring while indoors and asleep (π_s) was calculated. In Rarieda, where remarkably endophilic human behaviour would raise the greatest concern that the proportion of exposure occurring indoors (π_i) would overestimate the true fraction of exposure directly preventable by an ITN while sleeping (π_s), there were modest differences between the estimates of π_i and π_s , with the latter estimated as 0.82 for *An. gambiae s.l.* and 0.92 for *An. funestus s.l.*, whereas the former was estimated to be 0.95 and 0.97, respectively (Figure 3). Similarly, the proportions of human exposure to mosquito bites occurring while asleep in Luangwa were also high, being 0.77 for *An. gambiae s.l.* and 0.86 for *An. funestus s.l.*, as compared with values of π_i of 0.89 and 0.92, respectively.

Examining the binomial estimates of the proportion of human exposure occurring indoors (π_i^B) in the context of its two explanatory quantities (P_i and P_{II}) clearly shows even greater consistency across all of these *Anopheles*–human population interactions (Table 2). Consistent with estimates obtained by weighting indoor and outdoor vector biting rates according to the proportion of humans in those categories (Figure 3), the simple binomial estimates described in Table 2 indicate that almost all human

exposure to members of the *An. gambiae* complex and the *An. funestus* group occurred indoors ($\pi_i^B = 0.79$ – 1.00 and 0.88 – 1.00 , respectively). Interestingly, mosquito preferences for feeding indoors did not appear to be a strong driver of this epidemiologically crucial quantity,^{22–26} with P_i ranging from 0.40 to 0.63 for *An. gambiae s.l.* and from 0.22 to 0.69 for *An. funestus s.l.* Although *An. gambiae s.l.* populations in Luangwa, Ulanga, Korouwego, and Oubritenga, and *An. funestus s.l.* populations in Rarieda and Luangwa, exhibited clear preferences for feeding indoors ($P_i > 0.5$), the magnitudes of these preferences were modest and cannot explain the high values for π_i^B in these sites. Furthermore, human exposure to *An. funestus s.l.* at both the Kourowego and Oubritenga sites in Burkina Faso occurred mainly indoors ($\pi_i^B = 1.00$) despite the apparent preference of these vector populations for feeding outdoors ($P_i < 0.5$).

In stark contrast, estimates for the proportion of mosquitoes caught between the first and last hour when most humans were indoors (P_{II}) were consistently high, ranging from 0.78 to 1.00 for *An. gambiae s.l.* and from 0.86 to 1.00 for *An. funestus s.l.* (Table 2). In the absence of any evidence for strong mosquito preference for feeding indoors in the strict sense ($P_i \gg 0.5$), it appears to be the ubiquitously strong preference for feeding at times of the night when most humans are indoors ($P_i \geq 0.78$) that primarily drives the consistently high proportion of human exposure that occurs indoors ($\pi_i^B \geq 0.79$) across Africa.

Discussion

Apart from a scope that spans only six sites in four countries, this study has a number of limitations relating to its technical methodology. Previous comparisons of questionnaires with direct observations for surveying the human behaviours described here suggest that these are approximately but not entirely consistent with each other.³⁵ In terms of mosquito behaviour, several of the sites in the study may not have captured some low levels of outdoor human exposure that occurred before HLC surveys that were begun in the evening and after they ended in the morning. The proportion of human exposure occurring indoors (π_i) may therefore have been slightly overestimated. However, examining the trends on either end of the activity profiles in Figure 3 reveals that in no case is this likely to result in overestimation by more than 10%. Additionally, the accuracy of these mosquito surveys is limited to some extent by the practical challenge of maintaining consistently sensitive human landing catches throughout the night.²⁸ Lack of explicit molecular data for distinguishing sibling species and molecular forms within the major vector taxa occurring in both Tanzania and Burkina Faso also introduces ambiguity into the interpretation of the results of the study. This limitation is of greatest significance for the Burkina Faso sites, where both populations of *An. funestus s.l.* exhibited early peaks of

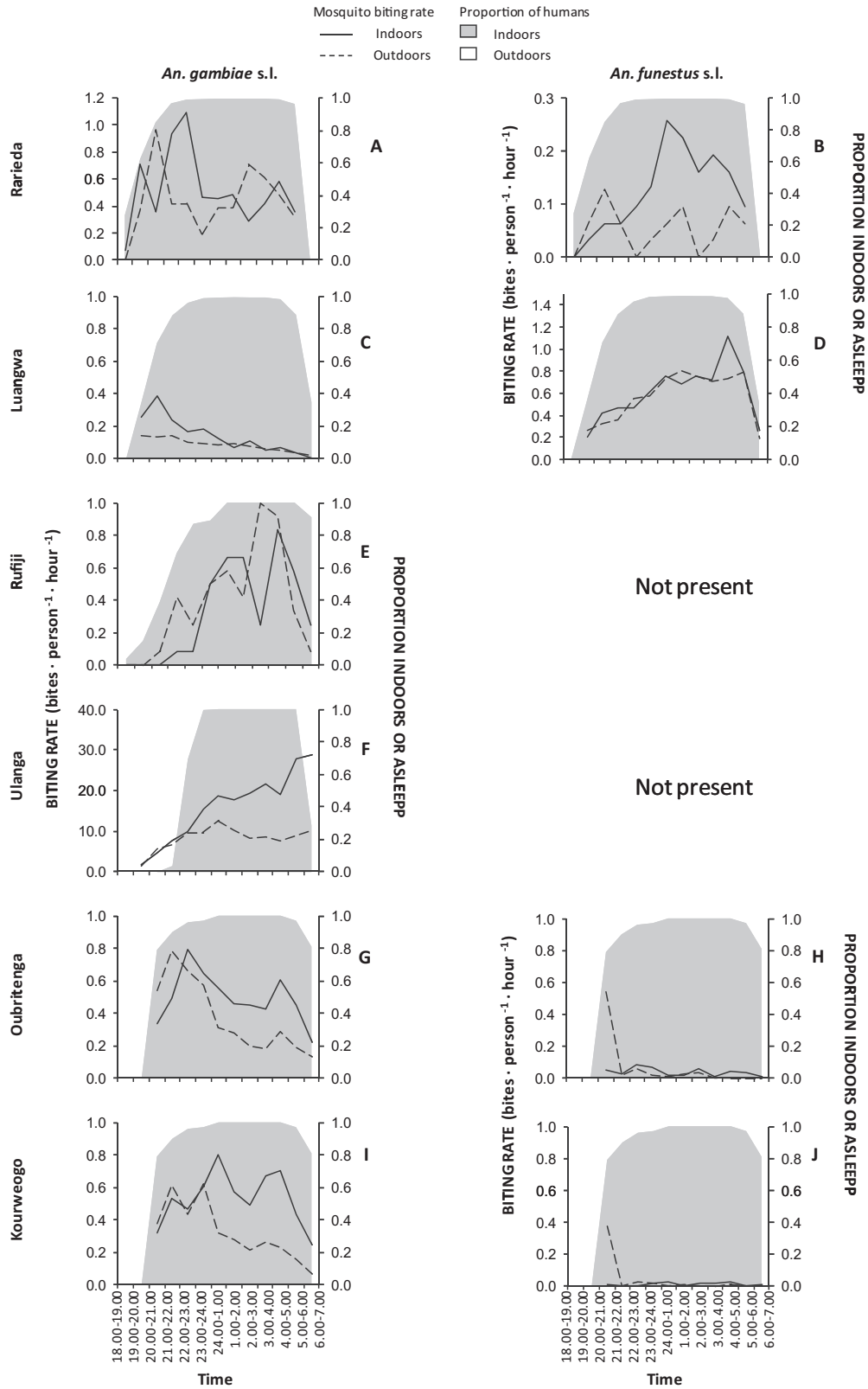


Figure 2 Hourly biting pattern of *Anopheles gambiae sensu lato* (panels on left) and *Anopheles funestus sensu lato* (panels on right) occurring both indoors (solid lines) and outdoors (dashed lines) at different study sites. The grey area represents the proportion of the human population predominantly spending time indoors during the times shown on the abscissa of each graph

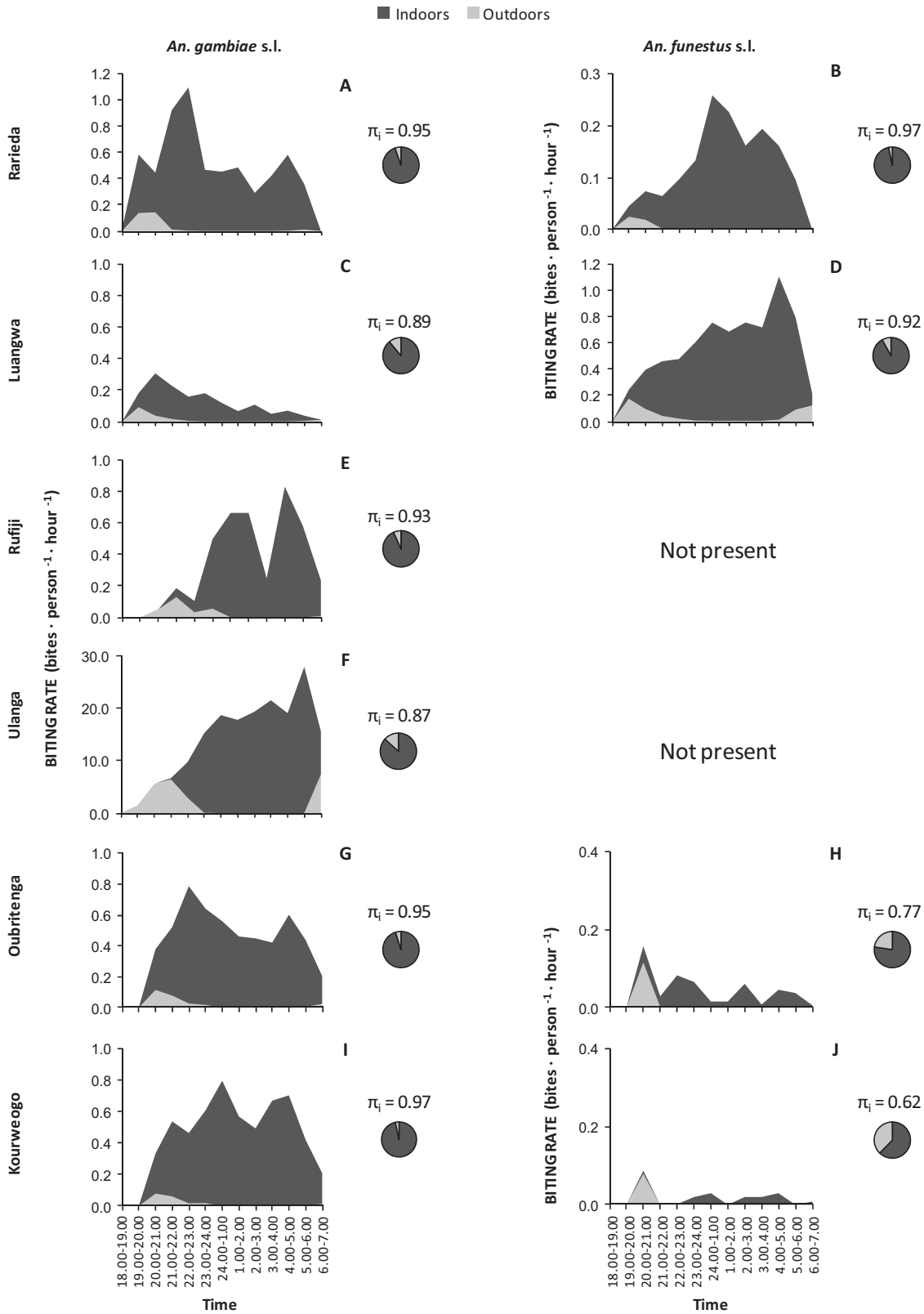


Figure 3 Profiles of biting by both *Anopheles gambiae sensu lato* (panels on left) and *Anopheles funestus sensu lato* (panels on right) experienced by human population at different study sites. Pie charts illustrate the amount of exposure occurring indoors and outdoors. The light grey areas in the graphs and pie charts represent outdoor human exposure and the dark grey areas represent indoor human exposure

Table 2 Estimates of proportion of mosquitoes caught indoors (P_I), proportion of mosquitoes caught between the first and last hour when most humans were indoors (P_N), and proportion of human exposure to mosquito bites occurring indoors, calculated in a binomial fashion (π_I^B) with mosquitoes assigned into the two categories of *Anopheles gambiae sensu lato* and *An. funestus s.l.* at six sites in Africa

| | n^a | P_I (95% CI) | P-value | P_N (95% CI) | P-value | n^b | π_I^B (95% CI) | P-value |
|--------------------------|-------|--|---------------------|--|---------------------|-------|---|---------------------|
| <i>An. gambiae s.l.</i> | | | | | | | | |
| | | Overall effect of site: ($\chi^2 = 66.80$, df = 5) | <0.001 | Overall effect of site: ($\chi^2 = 29.49$, df = 3) | <0.001 | | Overall effect of site: ($\chi^2 = 18.20$, df = 3) | <0.001 |
| Rarieda | 337 | 0.54 (0.48, 0.59) | 0.174 ^c | 0.78 (0.73, 0.82) | <0.001 ^c | 187 | 0.79 (0.72, 0.84) | <0.001 ^c |
| Luangwa | 638 | 0.63 (0.60, 0.67) | <0.001 ^c | 0.84 (0.81, 0.87) | <0.001 ^c | 380 | 0.90 (0.87, 0.93) | <0.001 ^c |
| Rufiji | 102 | 0.46 (0.36, 0.56) | 0.429 ^c | 0.99 (0.93, 1.000) | <0.001 ^c | 48 | 0.98 (0.87, 1.00) | <0.001 ^c |
| Ulanga | 320 | 0.40 (0.34, 0.45) | <0.001 ^c | 0.91 (0.88, 0.94) | <0.001 ^c | 127 | 0.89 (0.82, 0.93) | <0.001 ^c |
| Oubritenga | 1377 | 0.57 (0.55, 0.60) | <0.001 ^c | 1.00 ^d | <0.001 ^c | 791 | 1.00 ^d | <0.001 ^c |
| Kourweogo | 1019 | 0.62 (0.59, 0.65) | <0.001 ^c | 1.00 ^d | <0.001 ^c | 637 | 1.00 ^d | <0.001 ^c |
| <i>An. funestus s.l.</i> | | | | | | | | |
| | | Overall effect of site: ($\chi^2 = 44.19$, df = 4) | <0.001 | Overall effect of site: ($\chi^2 = 5.18$, df = 1) | 0.023 | | Overall effect of site: ($\chi^2 = 2.91$, df = 1) | 0.08 |
| Rarieda | 71 | 0.69 (0.57, 0.79) | 0.003 ^c | 0.86 (0.75, 0.93) | <0.001 ^c | 48 | 0.88 (0.75, 0.94) | <0.001 ^c |
| Luangwa | 3384 | 0.52 (0.50, 0.53) | 0.050 ^c | 0.93 (0.93, 0.94) | <0.001 ^c | 1746 | 0.94 (0.93, 0.95) | <0.001 ^c |
| Oubritenga | 155 | 0.36 (0.29, 0.44) | <0.001 ^c | 1.00 ^d | <0.001 ^c | 56 | 1.00 ^d | <0.001 ^c |
| Kourweogo | 62 | 0.22 (0.14, 0.34) | <0.001 ^c | 1.00 ^d | <0.001 ^c | 14 | 1.00 ^d | <0.001 ^c |

n , number of mosquitoes included in each analysis; P -value, estimated probability of the null hypothesis for each analysis; df, degrees of freedom.

^aTotal number of mosquitoes caught.

^bTotal number of mosquitoes sampled in the evening outdoors, at night indoors and in the morning outdoors.

^cEstimated probability for the null hypothesis of a value equal to 0.5.

^dConfidence interval could not be determined, as all mosquitoes were in one category.

outdoor biting activity (Figure 2H and J) that contrast clearly with historical observations of peaks in feeding activity that occurred indoors during sleeping hours for both *An. gambiae sensu stricto* and *An. funestus sensu stricto* in other areas of Burkina Faso.^{4,36} In the absence of molecular data with which to distinguish exactly which members of the *An. funestus s.l.* group contributed to these observations, we can only conclude that these distinct, early peaks of outdoor exposure may well be accounted for by secondary vectors, such as *An. rivulorum* or *An. parensis*,^{11,37–39} that can replace *An. funestus sensu stricto* when selective pressure is applied by vector control but are of negligible relevance to malaria transmission.^{5,15,40} This phenomenon also helps explain the discrepancy between the behaviour-weighted (Figure 3) and simpler binomial estimate (π_i^B , Table 2), and suggests that the latter may be more representative of exposure to important primary vectors: The more subtle weighted estimate captures the brief but intense period of largely outdoor exposure of a minority of residents to these peaks of biting activity by presumably secondary vectors that occurs between 20:00 and 21:00 hours (Figure 2).

However, the most important limitations of this study are fundamental in nature and relate to the relevance of the π_i parameter itself. Estimates of the proportion of mosquitoes that make contact with treatable surfaces while resting within houses (π_r) would be far more directly relevant to community-level transmission control with IRS rather than ITNs, but field methods for measuring such a quantity have yet to be developed. Also, the proportion of exposure occurring during sleep (π_s) is a more directly relevant determinant of protection with ITNs than is π_i , but could only be estimated for the Kenyan and Zambian sites. The assumption that the latter only modestly overestimates the former obviously introduces some degree of systematic inaccuracy. Examining the two sites for which both quantities are estimable suggests quite modest differences between these alternative estimates of individual protective coverage. However, a very different picture emerges when the same estimates are considered in terms of the gaps in protective coverage that allow malaria transmission and vector populations to persist, highlighting the crucial importance of high biological coverage against the transmission of malaria and accurate ways of measuring such coverage.³³ In Rarieda, biological coverage gaps of only 5% for *An. gambiae s.l.* and 2% for *An. funestus s.l.* are apparent when calculated as the complement of π_i , but this contrasts dramatically with values of 18% and 8%, respectively, for the complement of π_s . In Luangwa, corresponding coverage gaps of 11% and 8% when estimated on the basis of π_i are approximately doubled to 23% and 14% when based on the π_s measurement, which more accurately reflects protective coverage with nets.

Despite these limitations, a number of clear, useful, and broadly applicable conclusions can nevertheless

be drawn from the present study. It appears that the mosquito taxa that are responsible for most of the malaria transmission in Africa have only mild and inconsistent preferences for feeding indoors. However, biting contact with humans overwhelmingly occurs indoors simply because this is where people spend most of the hours of darkness, which is when these vectors are active. These findings are consistent with the long-standing rationale for prioritization of ITNs and IRS for malaria prevention in Africa, and support their continued upscaling across the continent.^{41,42} However, some human exposure to vector mosquitoes occurred outdoors in all sites in the present study (Figure 3), suggesting that additional vector control measures⁴³ that complement ITNs and IRS by targeting this gap in *de facto* protective coverage may well be required if malaria transmission is to be eliminated in such settings.²⁴

It is particularly encouraging that most of the human–vector interaction found in the present study occurred indoors in the most recently surveyed Rarieda and Luangwa sites, both of which had high ITN coverage at the time. In the Rarieda site, ITNs have had a clear^{44,45} and sustained^{46–48} impact on malaria transmission and malaria-related morbidity and mortality. Furthermore, substantive changes in vector population composition have occurred, with *An. gambiae s.s.* all but disappearing, leaving *An. arabiensis*, which is known to be capable of feeding extensively on humans early in the evenings, before humans go indoors,^{13,26,49} as the only remaining vector species of the *An. gambiae s.l.* complex.¹⁴ The continued high proportions of human exposure to transmission occurring indoors in the absence of personal use of an ITN (π_i) up to at least 2009 may well help explain why supplementing ITNs with IRS confers additional incremental protection in a nearby district,²⁷ despite dramatic changes in vector population composition, and may underpin similar observations elsewhere.⁵⁰

However, these continued high proportions of human exposure to bites by extensively modified residual vector populations¹⁴ in Rarieda contrast strongly with recent observations of dramatic declines in this proportion following ITN and IRS scale-up in Equatorial Guinea²⁰ and in the Ulanga site itself,¹⁹ as well outside of Africa in the Solomon Islands.¹⁸ It is therefore clear that summary estimates of relevant mosquito–human interaction quantities, such as P_i , P_n , and π_i , should be regularly monitored by national malaria control programmes and carefully considered by policy makers, product manufacturers, and public health funding bodies.^{23–25} Care should be taken not to misinterpret such reports of declining proportions of human exposure occurring indoors: These measurements do not necessarily reflect a failure of ITNs or IRS. Instead, they often represent the characteristics of persisting populations of zoophagic and exophagic mosquitoes following the successful control^{5,13–15,19,20,40,51} and even

elimination¹⁸ of anthropophilic and endophilic vector populations by ITNs or IRS. By definition, less anthropophilic mosquitoes are less efficient vectors of these malaria parasite species because *Plasmodium falciparum* and *P. vivax* are strict anthroponoses that only infect human hosts. Indeed many of these anthropophilic mosquitoes, such as the *An. quadriannulatus*, *An. rivulorum*, *An. parensis*, *An. vaneedeni*, and *An. lesoni* found in Luangwa, are considered to play a negligible role in sustaining the transmission of malaria.^{11,37} It may therefore be inappropriate to judge the ongoing effectiveness of commonly used vector control measures such as ITNs on the basis of contemporary measures of mosquito-human interactions because these measures reflect the characteristics of the surviving mosquito populations only. Quantitative estimates of mosquito behavioural parameters, such as those presented here (Figure 3, Table 2), collected before the scale up of ITNs or IRS (Ulanga, Kourowego, Oubritenga), or at least before these interventions had substantially reduced π_i values (Rarieda, Luangwa, Rufiji), may therefore be more representative than contemporary measurements for evaluating the ongoing impact of ITNs on vectors of historical importance. Such historical reference values are therefore crucial to balanced interpretation of contemporary estimates and observations of longitudinal trends. The consistency of the summary values presented in Table 2 suggests that it may be reasonable to extrapolate this range of values beyond the 6 study sites named above so that they may even constitute useful historical reference values for rural African vector populations generally.

Despite the limitations described in the two opening paragraphs of this discussion, measurements of π_i are very useful for approximately assessing *de facto* protective coverage of humans with ITNs and IRS.^{30,31,33} The proportion of human exposure to bites that occurs indoors can be most directly applied to estimating the maximum level of personal protection that can be realistically expected with indoor vector control measures, or combinations thereof.³⁰ However, the relevance of this behavioural parameter extends far beyond personal protection because it is critically important as a determinant of the greater community-level effects that ITNs and IRS can have when used by the majority of the population.³¹ Even though π_i does not directly reflect the probability of insecticide contact while resting, the high estimates for Rarieda help rationalise evidence for an incremental impact of IRS as a supplement to ITNs in a neighbouring district.²⁷ In Luangwa, similar estimates have been used to infer that IRS may also be a useful supplement to ITNs in that setting,³⁰ and the consistently high values presented here are in accord with recent reviews suggesting that this combination of malaria-preventive measures may have broad potential in Africa.^{50,52}

Beyond IRS and ITNs, π_i is also informative as a primary determinant of target product profiles for

complementary measures designed to fill the coverage gaps created when mosquitoes feed outdoors.^{24,33} It has long been recognized that pre-existing behavioural resistance traits, specifically preferences for feeding outdoors, usually limit the impact of vector control far more than does physiological resistance to the relevant active ingredients of insecticides.⁵³⁻⁵⁵ In fact, many of the diverse primary vectors distributed across tropical America and Asia are predominantly exophilic.⁵³⁻⁵⁷ Furthermore, residual mosquito populations that have persisted after the scale-up of ITN and IRS in Africa and the Pacific are often perfectly capable of mediating stable, endemic transmission of malaria because they include primary vectors that are behaviourally resistant to these measures.^{14,18-20,26,49,58,59} The primary parameter that determines the comparative merits of vapour-phase insecticides that can be used in outdoor spaces, as opposed to contact insecticides, which by definition require a treatable surface to which they can be applied, is the proportion of human exposure occurring indoors.^{24,33} The consistently high values for this quantity reported in Figure 3 and Table 2 confirm that ITNs and IRS using contact insecticides are indeed the logical first choices of intervention, while the intermediate values reported recently from residual populations across the tropics^{18,19,26} suggest that supplementary use of vapour-phase repellents may well effectively complement these traditional approaches in such situations.²⁴ In addition to the usual assays of physiological susceptibility to insecticides that are already integral to choosing vector control measures,^{21,60} up-to-date surveys of vector behavioural characteristics will also be essential to underpinning the selection of alternative or additional vector control technologies.

A recent modelling analysis cautions that supplementing existing ITNs or IRS with indoor use of spatial repellents may undermine and reverse the impact of the former upon historically important anthropophilic and endophilic vectors that have been suppressed but persist and can therefore recover if they are deterred from houses where they would otherwise be killed.²⁴ When deciding about whether to supplement ITNs with IRS, it is essential to consider not only the contemporary values of such behavioural quantities for surviving residual vector populations, but also the normal range of values for historically important vectors that need to be suppressed indefinitely.³³ To conclude, we believe that historical values for such behavioural parameters, recorded before the occurrence of wholesale changes in vector population composition, are likely to be more useful for rationalizing the impact of ongoing interventions, while equivalent, contemporary surveys of surviving residual populations are more appropriate for informing strategies to augment existing means of vector control and for ultimately eliminating the transmission of malaria.³³

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KEY MESSAGES

- African malaria vectors have no strong or consistent preference for feeding indoors.
- Nevertheless, most human exposure to biting malaria vectors occurs indoors because that is where humans sleep during peak hours of vector feeding activity.
- Mosquito feeding patterns should be monitored longitudinally to enable rational management of vector control programmes and guide the optimal formulation of target product profiles for new malaria-control technologies.

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Commentary: Does a mosquito bite when no one is around to hear it?

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Recent declines in malaria-related morbidity and mortality in Africa have been attributed in part to the widespread scale-up of measures for malaria control, including insecticide-treated bednets (ITNs) and indoor residual spraying (IRS). The percentage of households owning an ITN has increased from 3% to 50% in the past decade, and the number of households protected by IRS has more than quintupled during this same period, reaching 11% of the population at risk.¹ Both ITNs and IRS are particularly effective at reducing the transmission of malaria because they exploit the indoor (endophagy) and nighttime (nocturnality) biting and indoor resting (endophily) characteristics of the most efficient African *Anopheles* malaria vectors. In this way, ITNs and IRS provide both direct personal protection against infective mosquito bites as well as indirect community protection resulting from overall decreases in mosquito abundance.

There has long been concern that deployment of insecticides on a scale as grand as the roll-out of ITNs and

IRS in Africa could promote the development of insecticide resistance as well as behavioural changes among *Anopheles* spp. mosquitoes, eventually undermining the continued effectiveness of ITNs and IRS.^{2–5} It is now increasingly evident that the accelerating target-site and metabolic resistance to pyrethroid insecticides found in malaria vector populations throughout Africa is in large part the result of efforts at malaria vector control.⁶ Whereas there are definitive examples of the ways in which insecticide resistance has adversely affected IRS programs,^{7,8} the impact of pyrethroid resistance on the effectiveness of ITNs for malaria control is not yet clear.^{9,10} Documented instances of changes in the vectors host seeking behaviours attributable to ITNs and IRS have resulted in increased outdoor biting^{11,12} and shifts in peak biting times,¹³ leading to concerns that these changes could allow transmission of the disease to be maintained even after ITNs or IRS have been fully scaled up.

However, Bernadette Huho and colleagues at six rural sites in Africa (two in West Africa, three in